HIV-1 Specific Cytotoxic T Lymphocyte (CTL) Responses in Pediatric Infection

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Background

Over the past decade, the number of HIV-1 infected infants and children has rapidly increased through the vertical (mother-infant) transmission of the virus. It is currently estimated that one thousand four hundred infants are newly infected each day throughout the world [UNAIDS/WHO Working Group on Global HIV/AIDS and STD Surveillance in collaboration with National AIDS Programmes]. Great interest has developed, therefore, in the development of effective strategies to prevent vertical HIV-1 infection.

Proposed strategies for the reduction of the vertical transmission of HIV-1 have primarily focused on perinatal antiretroviral prophylaxis. Treatment of pregnant women and their infants with the reverse transcriptase inhibitor, zidovudine, can profoundly reduce the risk of vertical HIV-1 transmission [Connor, 1994]. However, the cost and logistic intensity of this antiretroviral regimen renders it impractical for use in developing nations, where most pediatric infections occur. These strategies would also not prevent the vertical transmission of HIV-1 beyond the neonatal period (e.g., through breastfeeding). A safe and effective infant vaccine regimen, begun at birth, would therefore be more desirable and might also provide the basis for lifetime protection against HIV-1 infection. Improved understanding of the early infection process and the capability of the young human infant to generate HIV-1 specific immune responses are important for the development of such a vaccine.

The natural history of vertical HIV-1 infection and correlates of disease

In general, vertically-infected children experience more rapid disease progression than children infected at an older age or adults. Approximately 23% of vertically-infected children develop an AIDS-defining condition in their first 1–2 years of life ("rapid progressors") and an additional 17% will develop an AIDS-defining condition by 4 years of age [Newell, 1994]. The tempo of disease progression in the remainder ("nonrapid progressors"; 60%) is variable, although at most 10% of vertically-infected children survive to 8 years with intact CD4 counts and without symptoms of disease [Martin, 1996].

Potential viral and host determinants of infection outcome have been studied. Within weeks of birth, rapid increases in the plasma HIV-1 RNA copy number to 10^5 to 10^7 copies per milliliter of plasma have been documented [Palumbo, 1995; Shearer, 1997]. Plasma HIV-1 RNA levels only gradually decrease over the first 1 to 2 years of life [Palumbo, 1995; Shearer, 1997] and mean plasma HIV-1 RNA levels remain greater than 10^5 copies per milliliter of plasma through at least the third year of life. A continued reduction in plasma HIV-1 RNA (mean –0.2 to –0.3 log decline per year) independent of clinical or immunologic status and antiretroviral therapy has been observed in vertically-infected children through 6 years of age [McIntosh, 1996; Mofenson, 1997]. The reason for prolonged elevation of plasma HIV-1 RNA levels during the early years of vertical HIV-1 infection is unclear. It has been suggested that a larger pool of permissive host cells (activated CD4 T cells) in infancy and early childhood might explain this observation. While the observed decrease in plasma HIV-1 RNA levels (10 to 100-fold) exceeds the age-related 3-fold reduction in CD4 T cells observed over the same time period [McIntosh, 1996], this could be due, in part, to the production of many virions

by an infected cell. Infection at a time of reduced ability to generate effective virus-specific immune responses might also account for the apparent diminished control of viral replication in infancy and early childhood. Support for this hypothesis is provided by studies that suggest that host immune responses may be potent selective forces for diversification [Ganeshan, 1997; Wolinsky, 1996], along with studies that have documented limited viral diversification over the six months of life [Salvatori, 1997].

HIV-1 specific CTL and the pathogenesis of HIV-1 infection

Virus-specific CTL are important for the clearance of acute viral infection and suppression of viral replication in chronic infections though they likely act in synergy with other virus-specific or non-specific immune responses (Reviewed in [Oldstone, 1995]). In adult primary infection, HIV-1 specific CTL are among the earliest documented host immune responses [Koup, 1994]. In established infection, HIV-1 specific CTL have been demonstrated in the absence of virus-specific *in vitro* stimulation [Walker, 1987]. Additionally, an individual may generate HIV-1 specific CTL directed against multiple structural (Envelope, Gag; reviewed in [Johnson, 1994]) or non-structural (Reverse Transcriptase; [Walker, 1988]), Nef [Riviere, 1994]) epitopes. Depletion or blocking studies indicate that HIV-1 Gag-specific CTL are CD8 T cell-mediated and HLA Class I restricted [Koup, 1989]. Studies of HIV-1 Envelope-specific cytolysis have demonstrated classic CD8 T cell-mediated, HLA Class I restricted responses as well as NK cell or neutrophil-mediated cytolysis through ADCC [Riviere, 1989].

The role of HIV-1 specific CTL in HIV-1 infection is unclear. The temporal association of the appearance of HIV-1 specific CTL with a reduction of blood viral load in adult primary viremia [Koup, 1994; Borrow, 1994; Borrow, 1997] and the association of high HIV-1 specific CTL precursor frequencies with preservation of CD4 counts [Greenough, 1997] suggest a protective role. However, chronic, high-level viral replication may continue despite a broad and vigorous HIV-1 specific CTL response [Luzuriaga, 1995].

Several factors may contribute to continued viral replication in the presence of an apparently vigorous HIV-1 specific CTL response. *In vitro* assay methods used to detect HIV-1 specific CTL may not detect biologically relevant CTL activity, i.e., high avidity CTL [Speiser, 1992]. Rapid and widespread dissemination of HIV-1 infection prior to the generation of CTL responses may "outstrip" the CTL response, particularly since effective neutralizing antibodies develop slowly in primary infection. Viral avoidance of CTL recognition may occur through replication in immune-privileged sites, induction of immunosuppression, the down regulation of MHC Class I or adhesion molecules on infected cell surfaces, or amino acid sequence variation (reviewed in [Koup, 1994]). Finally, viral-specific CTL, including HIV-1 specific CTL have been implicated in pathological processes [Jassoy, 1992], [Jassoy, 1993] and CTL responses may contribute to HIV-1 associated CD4 depletion or disease.

HIV-1 specific CTL in Vertical Infection

Virus-specific CTL responses have not been well-defined in children. Historically, murine models suggested that young mice were tolerized following fetal or neonatal antigen exposure [Oldstone, 1989]. More recent data indicate that young mice can generate CTL responses if antigen presentation is accompanied by appropriate co-stimulatory signals and if appropriate antigen doses are used [Ridge, 1996; Sarzotti, 1996; Forsthuber, 1996]. Cellular cytotoxic responses have been studied in human infants with acute respiratory syncytial virus (RSV) infection. After virus-specific *in vitro* stimulation, Isaacs and colleagues [Isaacs, 1987] detected RSV-specific, cell-mediated cytotoxic responses in 4 of 22 infants studied. Chiba and colleagues [Chiba, 1989] described the detection of age-dependent RSV-specific cellular cytotoxic responses, again after virus-specific *in vitro* stimulation. In these studies, however, effector cells and HLA restriction of these responses were not definitively characterized.

The large numbers of infants born to HIV-1 infected women each year has provided a unique opportunity to study the ability of infants children to generate virus-specific CTL, to characterize the CTL responses, and to evaluate their potential role in disease pathogenesis. HIV-1 specific CTL responses were first studied in older children with established disease. In an early study [Luzuriaga, 1991], activated Gag-specific HIV-1 specific CTL (i.e., those detected using freshly-isolated peripheral blood lymphocytes as effectors) were less commonly detected in vertically-infected children than in hemophilic children infected after the age of 2 years or in HIV-1 infected adults studied by the same laboratory [Koup, 1989]. A subsequent study [Buseyne, 1993] confirmed the less frequent detection of activated CD8 T cell-mediated, HIV-1 Gag and Pol-specific CTL in children compared with adults; direct Envelope-specific cytotoxicity was detected in the peripheral blood of 12 (51%) children of 21 studied but the responsible effector cell(s) were not fully characterized. In a third study [McFarland, 1994], circulating, activated HIV-1 Gag and Pol-specific CTL were detected in only 1 of the 11 children studied; activated HIV-1 Envelope-specific cytotoxic responses were detected in 5 of the 11 children but characterization of effector cells suggested that these responses were NK cell-mediated.

While activated HIV-1 specific CTL have been uncommonly detected in the circulation of HIV-1 infected children, HIV-1 specific CTL precursors (CTLp) recognizing at least 1 HIV-1 gene product have been detected in the majority ($>$ 60%) of vertically-infected children using non-specific *in vitro* stimulation of PBMC [Buseyne, 1993; Froebel, 1994]. It must be noted that most of the children studied thus far have been older than 2–4 years of age at first evaluation and thus primarily represent nonrapid progressors. In fact, at least 2 cross-sectional studies have documented CTLp in higher proportions of nonprogressor children than in children with rapid disease progression [Van De Perre, 1992; Luzuriaga, Manuscript in Preparation]. Prospective studies of several cohorts are now in progress to examine the potential relationship between HIV-1 specific CTL and disease progression.

Quantitation of HIV-1 specific CTLp after non-specific *in vitro* stimulation has revealed HIV-1 Gag (50–630 CTLp per million PBMC) and Envelope (66–330 CTLp per million PBMC) specific CTLp frequencies similar to those measured in adults with established disease [Koup, 1991, 1994]. All CTL activity detected after *in vitro* stimulation in these studies has been CD8 T cell-mediated.

Little information is available on the fine specificity of HIV-1 specific CTL responses in children. Responses to 2 or more epitopes (within the same or different gene products) have been defined in most HIV-1 infected children studied thus far [Buseyne, 1993] ; Luzuriaga et al unpublished data]. Again, it must be emphasized that most of these studies have been carried out in older children with less rapidly progressing disease and detailed, prospective studies of the CTL repertoire, beginning in infancy, are necessary.

Several groups have prospectively evaluated HIV-1 specific CTL responses in early vertical infection. Circulating, activated HIV-1 specific CTL are rarely detected in the first year of life [Luzuriaga, 1995]. Of interest, HIV-1 specific CTLp have also been uncommonly detected in early infancy (i.e., $<$ 4–6 mo), but have been detected in the circulation of most HIV-1 infected infants by the end of the first year of life [Luzuriaga, 1995]. This contrasts with the frequent detection of HIV-1 specific CTLp in the peripheral blood of adults within weeks of primary infection [Koup, 1989; Borrow, 1994; Borrow, 1997].

Most vaccinia constructs used in assays to detect HIV-1 specific CTL express laboratory strain gene products. In a small cohort of young HIV-1 infected infants, the use of vaccinia vectors expressing Envelope sequences derived from first infant isolates allowed earlier detection of HIV-1 specific CTL than the use of vaccinia vectors expressing HIV-1 IIIB Envelope sequences [Pikora, 1997]. Again, these data contrast with the frequent detection of HIV-1 specific CTLp in the peripheral blood of adults within weeks of primary infection using vaccinia vectors expressing HIV-1 IIIB Envelope sequences [Koup, 1989; Borrow, 1994; Borrow, 1997], suggesting that type-specific responses might predominate in early infancy.

HIV-1 specific CTL have been detected in infants who acquired HIV-1 infection *in utero*, as well as in infants who acquired infection during birth [Luzuriaga, 1995]. The detection of HIV-1 specific CTLp in the cord blood of an HIV-1 infected infant suggest that the capacity to generate virus-specific CTL develops in fetal life although the exact timing is unclear. Recent murine studies suggest that the antigen presentation and co-stimulatory signals required for generation of a CTL response by an immature host are more stringent than those required in a mature host; given appropriate antigen presentation and co-stimulatory signals, however, neonatal mice appear capable of generating antigen-specific CTL responses [Ridge, 1996]. Data from studies of HIV-1 specific CTL in infants support this model, rather than the classic "self versus nonself" model, that would predict tolerance following fetal or neonatal exposure to antigen.

HIV-1 specific CTL in exposed, uninfected infants

In an effort to define immune responses which protect against the acquisition of HIV-1 infection, HIV-1 specific immune responses have been studied in cohorts of individuals who have not acquired infection despite high risk exposures. Several groups have reported the detection of cellular immune responses, including HIV-1 specific CTL, in uninfected children born to HIV-1 infected women. In the first group of studies, activated Envelope, Gag, and Nef-specific cytotoxic activities were repeatedly detected in the peripheral blood of 2 infants through 17–35 months of age [Cheynier, 1992]. In the second group of studies, activated HIV-1 specific cytotoxic T cell responses were detected in the peripheral blood of 6 (25%) of 23 uninfected infants born to HIV-1 infected women; age at detection ranged from 15 to 50 months [De Maria, 1994]. Since no other evidence of HIV-1 infection was detected in the peripheral blood of the infants/children, these studies raise the question of how such vigorous CTL responses could be maintained in the absence of antigenic stimulation. Using virus-specific stimulation, Rowland-Jones [Rowland-Jones, 1993] and colleagues have reported the detection of HIV-1 specific CTL of unspecified phenotype in an uninfected infant. At least 3 other groups, including our own, have been unable to detect these responses, even after virus-specific *in vitro* stimulation [Luzuriaga, 1991; Buseyne, 1993; McFarland, 1994; Luzuriaga, 1997]. Further analyses of exposed but uninfected infants and children are necessary in order to determine whether clearance of infection occurs and to examine virus-specific immune responses that are potentially associated with viral clearance. If HIV-1 specific CTL responses are detected, characterization of the responses (including the documentation of virus-specificity) would have important implications for neonatal vaccine development.

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